

## SUPPLEMENTARY DATA

### **Familial chilblain lupus due to a gain-of-function mutation in STING**

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## **SUPPLEMENTARY METHODS**

### **WHOLE EXOME SEQUENCING**

Genomic DNA was extracted from blood using the QIAamp DNA blood mini kit (Qiagen). Exome sequencing was carried out at the Next generation sequencing facility, Center for Regenerative Therapy, TU Dresden. The SureSelectXT2 Target Enrichment System (Agilent Technologies) was used for exome enrichment according to the manufacturer's instructions. Briefly, genomic DNA was sheared, end repaired, 3'-end adenylated, ligated to pre-capture indexing adaptors and amplified. Libraries were hybridized to the SureSelect Capture Library followed by capture using streptavidin beads and final amplification of captured libraries. The resulting exome libraries were pooled for 100 bp paired-end sequencing on an Illumina HiSeq 2000 system and sequenced with an average 50-fold coverage. Reads were aligned to the human reference genome (hg19) with pBWA (0.5.9-r32-MPI-patch2) and further processed using SAMtools and Picard[1]. Data were further analyzed using the GATK pipeline (v 2.6-5) according to the GATK Best Practices[2] and based on the latest releases of the ENSEMBL and dbSNP databases.[3,4]

### **SANGER SEQUENCING**

The coding exons and flanking intron sequences of the *TMEM173* gene (NM\_198282) were amplified by PCR using gene specific primers (Eurofins MWG Operon; Table S1) and sequenced in both directions using the BigDye Terminator v1.1 Cycle Sequencing Kit (Applied Biosystems) on an 3130xl Genetic Analyzer (Applied Biosystems). Data were analyzed using the Vector NTI® Software (Life Technologies).

**Table S1.** Oligonucleotides used for Sanger sequencing of *TMEM173* and RT-PCR.

Name	Forward (5' → 3')	Reverse (5' → 3')
Exon 3	CAGAGCCTCAGTCCCAGAAG	CATTTGGGAGGGAGTAGTAGAAATAG
Exon 4	ACTGCTGTAAACGGGGTCTG	ACTTGGCCAGAGCTTCTACCTC
Exon 5	CCTGAGCTGAGTACTGGGAGTG	TGTTTAGAAACACAAGAGGCTGTG
Exon 6	AATCAGGTGGGAGATAGGGTTAG	CAGTGAATTTTCATCAGTGCTTG
Exon 7	ACCTCCCAGGACTCTATCGTTAC	CATAGCCCCTTCTGACTCTTTG
Exon 8	ATCTTCCCAGCAAGTTCTCTAGG	CCTGTGGAAGGAAATAGCTCTG
GAPDH	GAAGGTGAAGGTCGGAGTC	GAAGATGGTGATGGGATTTTC
GAPDH_P	FAM-CAAGCTTCCC GTTCTCAGCC-TAMRA	

### QUANTITATIVE REAL TIME RT-PCR

Total RNA was extracted from peripheral blood mononuclear cells isolated by Ficoll density gradient centrifugation using the RNeasy Mini Kit (Qiagen) followed by DNase I digestion. RNA concentration was measured with a spectrophotometer (NanoDrop® ND-1000, PEQLAB). cDNA was derived from 500 ng total RNA. Gene expression was determined by quantitative real time RT-PCR using Taqman Universal PCR Master Mix (Applied Biosystems) on an ABI7300 and normalized to *GAPDH* expression. Target genes were analyzed using predesigned TaqMan probes for *IFNB* (Hs01077958\_s1), *IFIT1* (Hs01675197\_m1), *ISG15* (Hs01921425\_s1), *RSAD2* (Hs01057264\_m1) and *SIGLEC1* (Hs00988063\_m1). For oligonucleotides used for qRT-PCR of *GAPDH* see Table S1. Blood samples from 10 healthy donors served as controls. All samples were run in triplicates and each experiment was run at least twice. Data obtained from the family

members studied and from control samples were expressed relative to a single calibrator. Patient probes before and after treatment with tofacitinib were run in one assay. The relative mRNA expression value for each transcript is equal to  $2^{-\Delta\Delta Ct}$  corresponding to the normalized fold change relative to the average gene expression of the 10 controls. Comparison of Ct values of *GAPDH* mRNA measurements obtained from the 10 controls and 34 patients with type I interferonopathy and IFN signature (excluding the patients of this study) showed no difference in gene expression (online supplementary figure S4).

### **GENERATION OF TMEM173 MUTATIONS**

Human wild type TMEM173 cDNA was cloned in a pSPORT6 mammalian expression vector. The G166E and N154S mutations were introduced using the QuickChange Site-Directed Mutagenesis Kit (Agilent Technologies) and verified by sequencing.

### **cGAMP STIMULATION**

HEK293T cells were seeded in DMEM supplemented with 10 % FCS, 2 mM L-glutamine and 1 % antibiotics-antimycotics at  $12.5 \times 10^4$  cells per well and cultured for 48 h. Prior to transfection, media were changed to 190  $\mu$ l Opti-MEM (Life Technologies). 0.6  $\mu$ l polyethylenimine and 200 ng of STING plasmid were diluted in 60  $\mu$ l Opti-MEM, incubated at room temperature for 30 min and added to cells. After 6 h, 250  $\mu$ l Opti-MEM with 20 % FCS was added. Following cultivation for 24 h, cells were stimulated with cGAMP (2'3'-cGAMP, InvivoGen) by digitonin delivery[14]. Briefly, culture medium was replaced with 500  $\mu$ l of digitonin permeabilization solution (50 mM HEPES pH 7.0, 100 mM KCl, 3 mM MgCl<sub>2</sub>, 0.1 mM DTT, 85 mM sucrose, 0.2 % BSA, 1 mM ATP, 0.1 mM GTP, 10  $\mu$ g/mL digitonin) with or without 4  $\mu$ g/ml cGAMP. Cells were then incubated at 37 °C for 2 h. Following a washing step with DMEM, 500  $\mu$ l DMEM complete was

added and the cells were incubated for additional 6 h. Supernatants were used for IFN- $\beta$  reporter assay. Cells were lysed for Western blot analysis.

#### **IFN- $\beta$ REPORTER ASSAY**

IFN- $\beta$  was determined using HEK-Blue IFN- $\alpha/\beta$  cells (InvivoGen).  $3 \times 10^5$  cells per well were seeded in a 24-well plate into 450  $\mu$ l of the supernatants from the cGAMP stimulation and cultured for 24 h. 20  $\mu$ l of induced HEK-Blue IFN- $\alpha/\beta$  supernatant was mixed with 200  $\mu$ l QUANTI-Blue™ detection medium, which detects and quantifies secreted embryonic alkaline phosphatase (SEAP), in a 96-well plate and incubated for 30 min at 37 °C. SEAP levels were measured with a photospectrometer (Infinite M200, Tecan) at 655 nm.

#### **WESTERN BLOT ANALYSIS**

HEK293T cells were lysed in RIPA buffer (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 1 mM EDTA, 1 % Triton X-100, 1 mM sodium orthovanadate, 20 mM sodium fluoride) supplemented with 1x Complete Protease Inhibitor Cocktail, 1x PhosSTOP phosphatase inhibitors (Roche) and Dnase I (Qiagen). Protein concentration was determined using a BCA Kit (Thermo Scientific). Lysates were resolved in a 4-12 % NuPAGE Bis-Tris gel under reducing and denaturing conditions and blotted onto a nitrocellulose membrane (BA83, Sigma-Aldrich). Membranes were blocked in 5 % dry milk and probed using the following antibodies: anti-pIRF3 (Ser386, ab76493, abcam; 1:1000), anti-STING (Cell Signaling; 1:1000) and anti-GAPDH (Meridian Life Science; 1:5000). Immunoreactive signals were detected by chemiluminescence (Lumi-Light PLUS, Roche).

## **HISTOLOGY AND IMMUNOHISTOCHEMISTRY**

Paraffin-embedded skin biopsies were cut into 2-5  $\mu\text{m}$  sections, rehydrated and boiled in sodium citrate buffer (pH 6.0). Sections were stained with Mayer's hematoxylin eosin (Merck). For immunohistochemistry, tissue sections were stained with mouse-anti-MxA (kindly provided by Otto Haller; 1:400), followed by staining with EnVision™ G|2 System/AP Rabbit/Mouse (Dako). Sections were counterstained with Mayer's hematoxylin (Merck) and analyzed with a Zeiss Axio Imager A1 microscope.

## **HOMOLOGY MODELING**

The 3D model of the hSTING mutant (G166E-hSTING) was predicted by MODELLER 9v15 software[5,6]. Homology modeling was based on the X-ray diffraction acquired high-resolution crystal structure of the wild type hSTING[7]. The FASTA sequence and crystal structure of the template protein were extracted from the Protein Data Bank[8] (PDB code: 4EMU). The best hSTING mutant model was selected using the MODELLER objective function known as the DOPE assessment score (discrete optimized protein energy)[9].

## **DOCKING SIMULATIONS**

Docking simulations of G166E-hSTING chains were performed with the program HADDOCK v2.1[10,11]. The modeled structures were clustered with a cutoff value of 6.0 Å for the pairwise backbone RMSD (root-mean-square deviation) matrix. Glutamic 166 and Threonine 263 of both monomers were selected as the active residues involved in the HADDOCK ambiguous interaction restraints (AIRs). The docking process is governed by the combination of electrostatic, van der Waals, and AIR restraints defined as "HADDOCK score". A total of 1000 docking solutions were generated during the rigid-body energy minimization step, and the best 200 were further subjected to the semi-flexible simulated annealing and final refinement in

explicit solvent. The resulting orientations, adopted by the dimer in the various complexes, represent an ensemble of structures most likely assumed in solution.

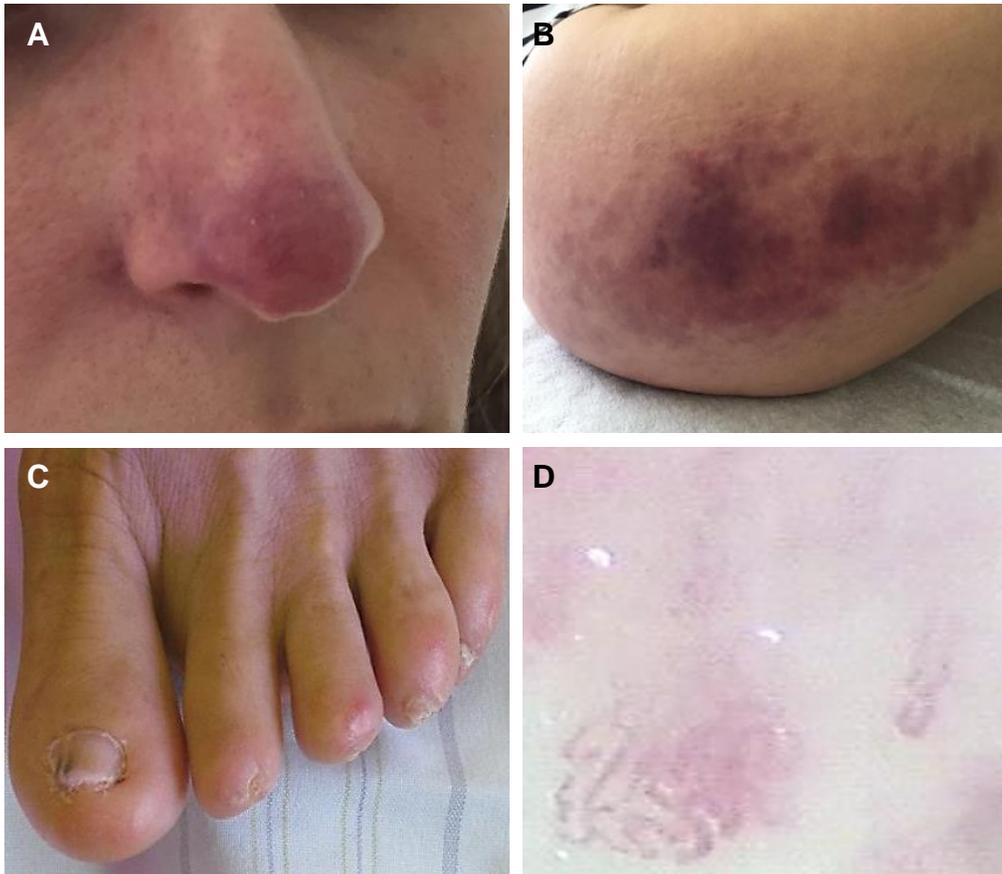
#### **ANALYSIS OF INTERFACE RESIDUES AND POLAR / NON-POLAR INTERACTIONS**

Residues involved in the interface of hSTING monomers were determined by identifying those in which the difference between dimer-based areas and monomer-only-based areas is greater than 1 square Angstroms. Polar and non-polar interactions along the hSTING dimer interface were computed by using LigPlot+[12,13] with molecular 3D visualizations performed in PyMol[14].

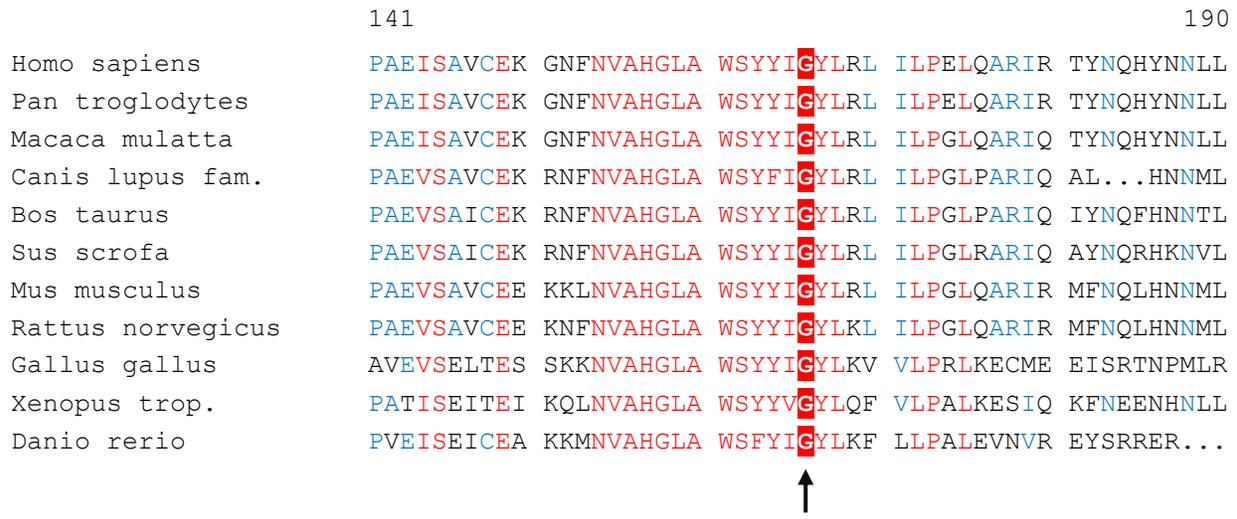
#### **TREATMENT OF PATIENTS WITH TOFACITINIB**

With written informed consent, two patients were treated with tofacitinib at a dose of 5 mg twice daily per os over 17 days. For assessment of IFN-stimulated genes, blood samples were collected 3 to 4 hours after the first dose of 5 mg tofacitinib at day 14 of treatment. As tofacitinib is not reimbursed in Germany for the indication of chilblain lupus, treatment was stopped after 17 days. We are currently awaiting approval of assumption of costs of further treatment by the patients' health insurance."

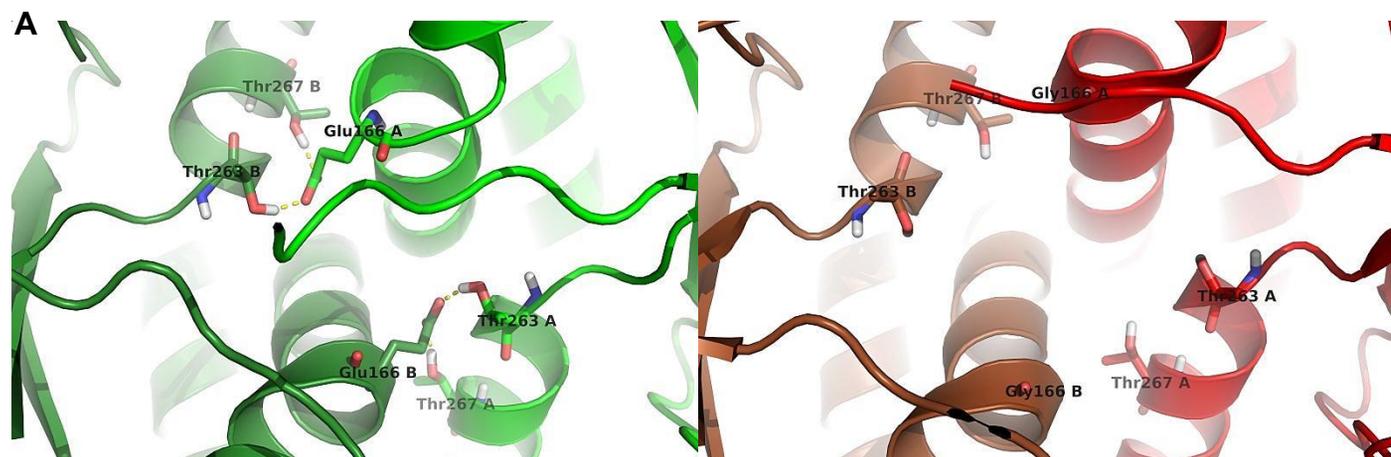
## SUPPLEMENTARY FIGURES



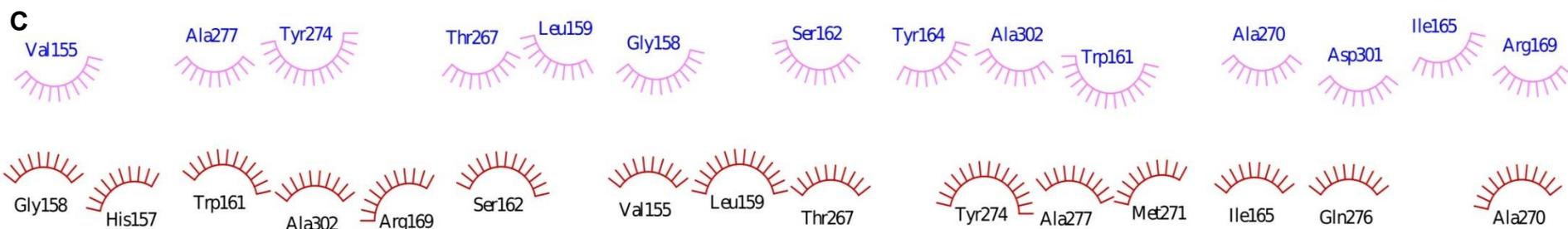
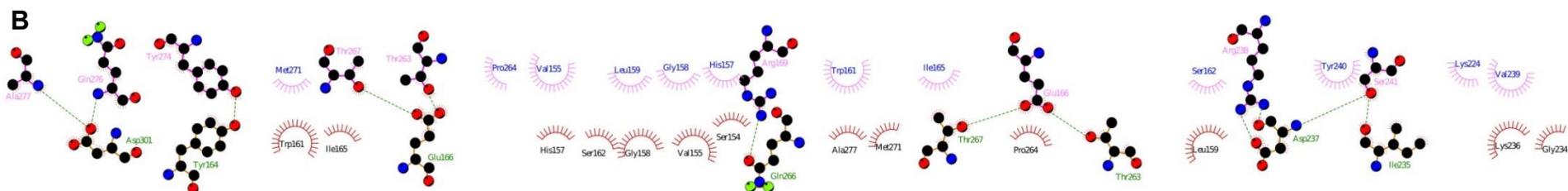
**Figure S1.** Cutaneous findings in family with chilblain lupus. (A, B) Erythematous to violaceous inflammatory skin lesion on the tip of the nose and the lateral side of the upper right thigh of individual III.2 at 32 years of age. (C) Reduction deformities along with onychodystrophia and nail loss of toes due to recurrent ulcerations. (D) Nailfold capillaroscopy of individual II.1 showing irregular distribution of capillaries with bushy appearance and enlarged loops.

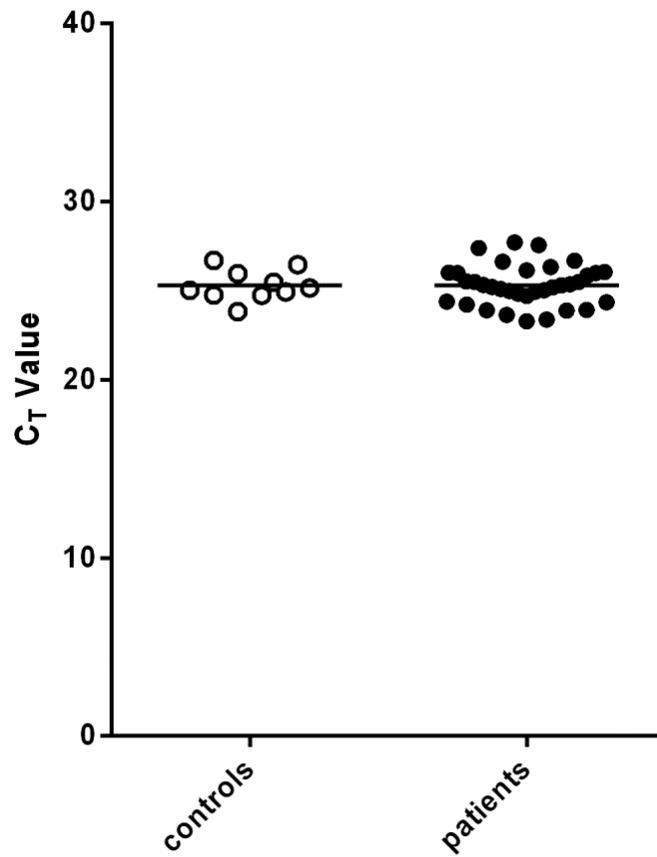


**Figure S2.** Multi-species alignment of STING. Alignment of STING homologs from different species. The numbering refers to the human STING protein sequence. The position of the newly identified mutation G166E is marked by an arrow. It is located within the highly conserved dimerization domain of STING. Homologues were retrieved from the ENSEMBL database[3] and were computed with multiple sequence alignment with hierarchical clustering[15]. Highly conserved regions are marked in red and less conserved regions are indicated in blue.



**Figure S3.** Gly166Glu triggers new intramolecular polar interactions at the STING dimer interface. (A) Close-up of mutant (green) and wild type (red) hSTING dimer. Glu166 induces formation of hydrogen bonds with Thr263 and Thr267 (sticks) of the contralateral monomer, which are absent in the wild type dimer. (B) Two-dimensional LigPlot+ diagram depicting multiple polar contacts along the dimer interface produced by Glu166. (C) LigPlot+ diagram of wild type dimer interface showing only non-polar interactions.





**Figure S4. Validation of normalization of gene expression.** Comparison of Ct values of *GAPDH* mRNA measurements obtained from 10 controls (open circles) and 34 patients with type I interferonopathy and IFN signature (black circles) showed no difference in gene expression ( $\Delta C_t$  ref [mean control – mean sample] = -0,01). All samples were run in triplicates. Data were expressed relative to a single calibrator. Horizontal lines indicate means.

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